Hemostatic efficacy of pathogen-inactivated- versus untreated- platelets: a randomized controlled trial

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- Pathogen-inactivated platelets were noninferior in preventing bleeding only in intention-to-treat analysis
- In contrast to animal models, alloimmunization could not be prevented when using pathogen-inactivated platelets

ABSTRACT

Pathogen inactivation of platelet concentrates reduces the risk of blood-borne infections. However, its effect on platelet function and hemostatic efficacy of transfusion is unclear. We conducted a randomized noninferiority trial comparing the efficacy of pathogen inactivated platelets using riboflavin and ultraviolet B illumination technology (intervention) compared to standard plasma-stored platelets (control) for the prevention of bleeding in patients with hematologic malignancies and thrombocytopenia. The primary outcome parameter was the proportion of transfusion treatment periods in which the patient had grade 2 or higher bleeding as defined by World Health Organization (WHO) criteria. Between November 2010 and April 2016, 469 unique patients were randomized to 567 transfusion treatment periods (283 in the control arm, 284 in the intervention arm). There was a 3% absolute difference in grade $\geq$ 2 bleeding in the intention-to-treat analysis: 51% of the transfusion treatment periods in the control arm and 54% in the intervention arm (95% CI -6 to 11, p-value for noninferiority 0.012). In the per-protocol analysis, however, difference in grade $\geq$ 2 bleeding was 8%: 44% in the control arm and 52% in the intervention arm (95% CI -2 to 18, p-value for noninferiority 0.19). Transfusion increment parameters were about 50% lower in the intervention arm. There was no difference in the proportion of patients developing HLA class I alloantibodies. In conclusion, the noninferiority criterion for pathogen inactivated platelets was met in the intention-to-treat analysis. This finding was not demonstrated in the per protocol analysis. (The Netherlands National Trial Registry number: NTR2106).

INTRODUCTION

There remains interest in development of pathogen inactivation techniques to complement the 'multi-layered prevention strategy' to avert transfusion of blood products contaminated with currently known as well as unknown pathogens. The available pathogen inactivation systems for platelet concentrates inactivate a broad array of viruses, bacteria and parasites.[1-4] Moreover, these techniques have also shown sufficient white cell inactivation to prevent transfusion-associated graft versus host disease, and may also reduce the formation of Human Leukocyte Antigen (HLA) antibodies.[5-7] If hemostatic efficacy of pathogen inactivated platelets is sufficiently maintained, these advantages could favor the consideration to implement pathogen inactivation technology. A meta-analysis of 12 randomized controlled trials concluded that transfusions with pathogen inactivated platelet concentrates resulted in reduced transfusion increment, without hemostatic consequences or differences in patient survival.[8] In three of these trials riboflavin, also known as vitamin B2, with ultraviolet illumination (Mirasol pathogen inactivation technology; Terumo BCT, Lakewood, Colorado) was used to inactivate pathogens.[9-11] Despite the available data, it is insufficiently known whether Mirasol treatment in platelet concentrates results in an equivalent hemostatic effect in this vulnerable population. As bleeding is considered to be the pivotal outcome for platelet transfusion trials, we conducted a non-inferiority randomized controlled trial comparing pathogen-inactivated platelet concentrates using the Mirasol technology with conventional untreated platelet concentrates, with percentage of transfusion treatment periods in which the patient has World Health Organization (WHO) grade ≥ 2 bleeding as primary outcome.[12] As a secondary outcome we measured HLA antibody-formation to determine whether pathogen-inactivated platelets are able to reduce alloimmunization in hemato-oncology patients.

METHODS

The PREPAReS study (Pathogen Reduction Evaluation and Predictive Analytical Rating Score) was designed as a randomized multicenter non-inferiority study using a parallel arm design with one-to-one randomization. The protocol was written by a steering committee and approved centrally and by site institutional review boards. The study met the criteria captured in the Declaration of Helsinki (6^{th} revision, 2008) and Good Clinical Practice guidelines. All patients gave written informed consent before the randomization procedure or any other study

related proceeding. A detailed review of the protocol and methods used was published separately, and is only briefly summarized here.[13] The study was conducted in three countries, in ten centers with hemato-oncology departments: 4 sites in the Netherlands, 5 in Canada and 1 in Norway.

Eligibility Criteria

Hemato-oncology patients with chemotherapy-induced thrombocytopenia aged 18 years or older were eligible for inclusion in the study if they were expected to require at least two platelet transfusions during a transfusion treatment period (Figure S1 in the supplementary appendix). Patients presenting with a grade ≥ 2 bleeding before enrolment could only be enrolled with existing (i.e. not new) bruises, while patients with grade ≥ 2 bleeding at other organ systems than skin could be enrolled only 14 days after resolution of the bleed. Other exclusion criteria included: known immunological refractoriness to platelet transfusions; indications to use hyperconcentrated platelets; idiopathic thrombocytopenic purpura (ITP); pregnancy; microangiopathic thrombocytopenia; known allergy to riboflavin or its photoactive products.

Stratification and Randomization

Eligible patients were randomized to receive untreated plasma-stored platelet concentrates or pathogen-inactivated platelet concentrates using a centralized, web-based allocation tool. The random allocation schedule was prepared by a biostatistician not directly involved in the study using a 1:1 ratio and randomly-varying block sizes of two to six. Three stratification factors were applied: center, diagnosis (AML vs. non-AML) and treatment (transplant vs no-transplant). Patients could be randomized more than once if they had subsequent hospital admissions, and the statistical analysis accounted for multiple randomizations per individual.

Platelet products and transfusion policy

Platelet concentrates were all prepared from pooled buffy coats, resuspended in plasma, and leukoreduced by filtration.[14] For pathogen inactivation, 35 ml (500 µM) riboflavin was added to the pooled leukoreduced product, and exposed to ultraviolet light (wavelength 280 – 315 nm) for 5 – 10 minutes depending on the volume of the concentrate (total dose 6.2 J/ml) according to the manufacturer's instructions. Platelet products were stored with gentle agitation at 20-24°C up to five days in Canada and for a maximum of seven days in the Netherlands and Norway.[14] The products were composed of five buffy coats in the Netherlands, four buffy coats in Norway, and four or five buffy coats in Canada. The actual platelet content in the bags largely overlapped between the countries. [14] An automated culture system was used to detect bacterial contamination, and products were issued as 'negative-to-date'. Platelet concentrates were γ-irradiated as per local protocol. In both treatment arms, patients received platelet transfusions prophylactically (platelet count-related prophylaxis, trigger $10x10^9$/L or intervention-related prophylaxis, trigger $50x10^9$/L) or as treatment of bleeding, using national and hospital guidelines. Red cell concentrates and plasma were transfused based on local protocols for transfusion thresholds and at the treating physicians' discretion.

Outcomes and clinical assessments

The primary study outcome was the proportion of transfusion treatment periods in which the patient had a bleeding complication WHO grade ≥ 2. The transfusion treatment period started at the time of the first platelet transfusion after randomization and ended maximally 6 weeks after the first platelet transfusion, or for one of the following reasons: patient was no longer thrombocytopenic (> 7 days without requiring a platelet transfusion), hospital discharge, death, or request by the patient to discontinue (Figure S1 in the supplementary appendix). Secondary outcomes were 1- and 24-hour corrected count increments, the frequency of transfusion failures (defined as 1-hour corrected count increment < 7.5 and 24-hour corrected count increments < 4.5), percentage of days within a transfusion treatment period with bleeding grade ≥ 2, incidence of adverse transfusion reactions, transfusion requirement of red cells and platelets, platelet transfusion interval, and the proportion of patients with HLA alloimmunization. Data collection was performed by trained research staff at each site, and data were entered into the ProMISe (Project Manager Internet Server) database from two central research locations in Canada and the Netherlands. Bleeding symptoms, as well as all

other clinical- and transfusion-related data were monitored daily on all study patients, starting at randomization, up to a maximum of 6 weeks after the first platelet transfusion, or end of thrombocytopenia as defined above. The study was not blinded, and bleeding assessments were performed by trained non-blinded research personnel. Hence, an adjudication process was used to assign each patients bleeding status to minimize bias. Bleeding adjudication, using the WHO bleeding scale, was done by three independent adjudicators blinded to the treatment allocation, in addition to the use of an automated algorithm.[15] For HLA antibody detection, samples were collected weekly during hospitalization up till day 28, and a 'late' sample at approximately day 56, and tested in the Luminex assay (Luminex Corp., Austin, Texas, USA) for presence of single antigen HLA-antibodies at the Blood Systems Research Institute (San Francisco, California, USA).[16]

Statistical analyses

A pilot study showed that on average 50% of patients have bleeding ≥ grade 2 during their thrombocytopenic phase, confirming findings of earlier large platelet transfusion studies.[17-19] The study was designed as a non-inferiority trial to test the null-hypothesis that pathogen-inactivated platelet concentrates are worse than control platelets. The alternative hypothesis to be proven is that the pathogen-inactivated platelets perform similar to control platelets within a pre-specified margin with regard to the primary endpoint. Based on discussions with clinicians as well as another large study using bleeding as an endpoint we decided that a 12.5 percentage point increase as the upper limit of the 95% confidence interval of the absolute difference in grade ≥ 2 bleeding between the treatment arms was an acceptable margin, acknowledging improved safety with regard to the transmission of pathogens.[17] To assess the non-inferiority hypothesis with a power of 80%, as well as adjustment (alpha and beta-spending) for predefined interim analyses required a sample size of 578 (289 per arm). For safety reasons, frequent interim analyses were performed after every 60 patient transfusion treatment period randomized using a flexible stopping rule based on alpha and beta spending functions, allowing stopping for non-inferiority or futility.[20] Before unblinding and starting the final analyses, a statistical analysis plan was written and agreed upon by the steering committee. The analysis of the primary endpoint as well as the majority of secondary endpoints were performed using three approaches: intention-to-treat (ITT), the per-protocol population (PP) and the per-protocol-only population (PPO) (Table S1 in the supplementary

appendix). The primary effect parameter was estimated according to a generalized estimating equation (GEE) approach using a generalized linear model with identity link and independence working correlation. The dependent variable was the yes/no indicator of having at least one ≥ grade 2 bleed during a transfusion treatment period. Covariates in the model were the treatment arm, the treatment period number (dichotomized as first or later) and the interaction between these two covariates. The 1-hour and 24-hour corrected count increments were analyzed using a linear mixed model using a random intercept per patient and a random intercept per treatment period to take into account the correlations between transfusions within treatment periods as well as between treatment periods within patients. Covariates were treatment arm, the number of the transfusion within the treatment period, the interaction between both and the pre-transfusion count. The platelet transfusion interval was analyzed with a mixed Poisson model with the number of transfusions per treatment period as dependent variable, the treatment arm as covariate, the log of the duration of the treatment period as an offset parameter and a random intercept per patient. The other numerical secondary outcomes that were measured only once per treatment period were compared based on the mean value per group with a similar GEE approach as for the primary outcome only now using a general linear model. For the analysis of the alloimmunization data, for patients with multiple randomizations, only results of the first randomizations were used. Patients were considered to be alloimmunized if at least one sample during 56 days after randomization had a signal higher than 5 standard deviations above the normalized background signal. We calculated Kaplan Meier curves for time-to-alloimmunization and compared both groups using a risk ratio for cumulative event probabilities estimated at 60 days. All statistical analyses were performed using IBM SPSS Statistics (version 23).

Study oversight

Safety aspects of the study were closely watched by a Data Safety Monitoring Board (DSMB). Interim analyses after every 60 randomized patients were evaluated by the DSMB. The study was monitored for quality and regulatory compliance. The monitoring frequency depended on inclusion rates and findings from earlier visits. The authors vouch for the integrity of the data and analyses reported. The study was sponsored by Sanquin Blood Supply and registered at the Netherlands National Trial Registry under number NTR2106, and also at clinicaltrials.gov under number NCT02783313.

RESULTS

From November 2010 until April 2016, randomization of 567 transfusion treatment periods took place in 469 patients. In November 2015, after analyzing 433 treatment periods the DSMB advised to stop recruiting patients, as analysis of the intention-to-treat population permitted a conclusion of non-inferiority for the primary endpoint. In close collaboration with the ethics review board, since there were no safety issues involved, the steering committee decided to continue patient accrual in order to reach the originally planned power of the study for the secondary endpoints, especially alloimmunization. Of the randomized transfusion treatment periods, 11 were excluded from further analyses as the patient had an active grade ≥ 2 bleeding (N = 8) at randomization, or there was a gross lack of study compliance (N = 3, Figure 1). The intention-to-treat analyses were thus performed on 556 transfusion treatment periods. For the per-protocol analyses the data set consisted of 425 treatment periods after excluding patients who actively bled on the day of the first transfusion or did not receive any transfusion or received ≥ 25% off-protocol transfusions (Figure 1). Randomization successfully balanced the most important risk factors for bleeding (Table 1).

Bleeding

In the intention-to-treat analysis, in 51% of the transfusion-treatment periods in the control arm the patient experienced a grade ≥2 WHO bleeding versus 54% in the intervention arm. The upper boundary of the 95% confidence interval of the difference between these two percentages did not exceed 12.5 percentage points, hence meeting the non-inferiority criterion (Table 2). However, for the per-protocol analysis, 44% of patients receiving standard platelet products had a grade ≥2 bleeding, versus 52% in the intervention arm (Table 2). The upper limit of the 95% confidence interval of this difference exceeded the prespecified limit, so the non-inferiority criterion was not met here (Figure 2). When looking at the percentage of bleeding days, there was no significant difference between the arms, irrespective of the analysis used. Also, when considering the highest bleeding grade, we saw no differences between the control and intervention arm. A further sub-analysis was performed for patients receiving only on-protocol transfusions, which showed similar outcome as compared to the per-protocol analysis (Table S2 in the supplementary appendix).

Transfusions

Most platelet transfusions were given prophylactically (Table S3 and S4 in the supplementary appendix). The pre-transfusion platelet count was about 15 x 10^9/L with no differences between the two arms. The platelet content in the products was about equal.[13, 14] (Table S3 and S4 in the supplementary appendix). Storage time was comparable, with 16 to 19% of the concentrates being stored for 6 or 7 days. The percentage of off-protocol transfusions in the intervention arm was 19.5% as compared to 11.6% in the control arm (p = 0.02). Off-protocol transfusions were denoted as "other", and could consist of -for example- hyperconcentrated platelet products; platelets in additive solution in the control arm, and untreated platelets in the intervention arm. All transfusion increment parameters were significantly lower for pathogen-inactivated platelet concentrates versus untreated platelets. In the intervention arm, the count increments and corrected count increments were about 50% lower than the values in the control platelets arm, resulting in frequent transfusion "failures" (Table 3), a higher number of platelet transfusions and a shorter platelet transfusion interval (Table 4). There were no differences in the number of red cell- and plasma-units transfused in either arm, for both intention-to-treat and per-protocol analysis (Table 4).

Safety: infections, (severe) adverse events, including transfusion reactions

There were a considerable number of infectious complications, adverse events (AEs) and serious adverse events (SAEs), without differences between both study arms (Table S5 in the supplementary appendix). In both arms, one SAE was related to the platelet transfusion, an anaphylactic transfusion reaction to an off-protocol transfusion of platelets in additive solution in the control arm, and a transfusion-associated lung injury in the intervention arm (imputability possible). The percentage of transfusion reactions with imputability probable, possible, or certain was 2.8% in the control arm and 3.3% in the intervention arm. The majority of the transfusion reactions in both arms resulted in no or only minor morbidity.

Alloimmunization

For the alloimmunization, we only included the first randomization transfusion treatment periods of patients (n=463). Excluding treatment periods with no or only one collected sample, as well as patients with HLA antibodies at the onset of their transfusion treatment period, resulted in 356 evaluable treatment periods in the per-protocol-only population (Control n = 177, Intervention n = 179). As shown in Figure 2, the number of patients developing HLA class I alloantibodies was similar: 6 in the control arm, as compared to 7 in the intervention arm (Risk ratio 1.00; 95% CI 0.34 – 2.98, p = 1.00). The intention-to-treat and per-protocol analyses are shown as supplemental material (Figures S2 and S3).

DISCUSSION

Using WHO bleeding as a primary outcome, we compared pathogen-inactivated platelet products using riboflavin and ultraviolet light, with standard plasma-stored platelet products in a multicenter, international randomized controlled trial using a non-inferiority design. The percentages of bleeding patients is in the same order of magnitude as other large randomized platelet transfusion trials, though somewhat higher as compared to the other two trials testing riboflavin/ ultraviolet light treated platelets, indicating that bleeding symptoms were accurately captured in the participating sites.[10,11, 17, 18] Although in the intention-to-treat analysis the non-inferiority criterion was met, the per-protocol analysis showed a slight increase in grade ≥ 2 bleeding complications in the intervention arm, and the upper limit of the 95% confidence interval of the difference crossed the margin of 12.5 percentage points. As has been recently discussed by Mauri and D'Agostino, in non-inferiority trials both the intention-to-treat as well as the per protocol analysis have important merits as well as pitfalls. Reporting both is considered to be the standard with similar results in both supporting the robustness of the findings.[21] In our study in the intention-to-treat analysis, both off-protocol transfusions as well as the inclusion of bleeding complications occurring between randomization and the first on study platelet transfusion likely resulted in a diluting effect to the advantage of the intervention arm. However, the per-protocol analysis might be hampered by selection bias. It is conceivable that excluding patients with active bleedings at the day of the first on-study transfusion resulted in a bias to the advantage of the control arm by removing patients with a bleeding tendency. A modified PP analysis, not excluding patients with active bleeding, reduced the difference with regard to bleeding complications between both populations slightly, though still not meeting the non-inferiority criterion.

With regard to secondary bleeding endpoints, there were no differences between both study arms. Importantly, though the numbers are small, no differences were observed in severe bleeding complications, pertinent to daily clinical practice. There were no differences with regard to the consumption of red cell concentrates or plasma, considered to be surrogate markers for clinically significant bleeding complications.

The small detrimental effect on hemostasis seen in de per protocol analysis is in concordance with the conclusions of the most recent Cochrane analysis on pathogen reduction as well as the outcome of the recently published EFFIPAP study, which compared amotosalen-ultraviolet A treated platelets with platelets in plasma as well as platelet additive solution.[8, 22] The observed increase in bleeding complications is likely due to the detrimental effects on platelet function induced by pathogen reduction as has been shown in vitro for all the currently available pathogen reduction techniques.[23,24]

All transfusion increment parameters were in favor of the control arm, which translated to a higher usage of platelet products in the intervention arm because the transfusion trigger is met sooner, with an increase of approximately 1 product per patient. This is as expected, recently published clinical studies comparing pathogen reduced platelet concentrates with untreated platelets also report higher platelet transfusion need.[11, 22] Possibly, the lower corrected count increments are also due to the effects on platelets induced by pathogen inactivation, described for several pathogen inactivation methods .[22, 25,26] This subject should be the basis for future research.

As expected in this population, there was a high number of adverse and serious adverse events, with only two serious adverse events related to a platelet transfusion. In the intervention arm a possible transfusion related acute long injury (TRALI) was reported. All platelet products in plasma can cause a TRALI, and since pathogen inactivation does not target proteins, such an occurrence is not unexpected. In contrast to recently published animal studies, pathogen inactivation treatment did not result in a reduction of HLA class I alloimmunization.[7, 27] As the percentage of immunized patients is low in both arms, this result may be completely explained by randomness. Additionally, the discrepancy between animal and human studies may be explained by the administration of untreated red blood cells in patients in both arms, which did not occur in the animal experiments. The recently published data of the IPTAS trial also reported comparable low rates of HLA class I antibodies.[28]

Numbers of countries, hospitals, patients, the large number of platelet transfusions and the large number of observed days are the main strengths of this study, contributing to the generalizability of conclusions regarding the clinical efficacy of pathogen-inactivated buffy-

coat platelets in thrombocytopenic hematology patients. Despite efforts to reduce this, the main weakness of our study is the significant number of patients with off-protocol transfusions. Since our study has shown a mildly reduced hemostatic efficacy as well as a significant impact on transfusion increments, to implement or not to implement pathogen-inactivated platelet products really depends on the balance of increased safety for known and unknown pathogens, which varies between countries worldwide, and the clinical effects that pathogen inactivation causes to the platelet product. Health-economic arguments should also be taken into account. Clearly there is room and need to improve the current techniques of platelet pathogen inactivation. Indeed replacing plasma by novel additive solutions has recently shown promising results.[29] Moreover a clinical trial using pathogen inactivation in apheresis platelets, potentially contributing to a decreased risk in alloimmunization, is about to start.

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AUTHORSHIP

Contribution: P.F.M., J.A.H., R.J.W.V., R.P.G., A.B., J.L.H.K. contributed to the conception and design of the study. P.F.Y., R.J.W.V., O.E., J.J.Z., M.T., E.A.B., P.B., A.T., Y.L., C.H., D.L., P.J.N., T.H., N.M.H. and J.L.H.K. contributed to the acquisition of data. P.F.M., P.F.Y., N.G. and J.L.H.K. analyzed and interpreted the data and developed the drafts for the manuscript. R.J.W.V., R.P.G., A.B. and J.G.B. contributed to the interpretation of the data. All authors revised the manuscript critically for content and gave final approval for the manuscript. All authors are accountable for all aspects of the work.

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Table 1 Patient characteristics

		Control n = 279	Intervention n = 277
Male/female	n / n	191 / 88	188 / 89
Age	Years, mean ± SD	54 ± 12	54 ± 12
Body surface area	m^2, mean ± SD	1.97 ± 0.25	2.00 ± 0.24
Enlarged spleen	n (%)	17 (6.1)†	31 (11)
Multiple inclusions	n (%)	57 (20)	39 (14)
Diagnosis			
Acute myeloid leukemia	n (%)	132 (47)	133 (48)
Acute lymphoblastic leukemia	n (%)	25(9.0)	24 (8.6)
Mantle cell lymphoma	n (%)	13 (4.7)	14 (5.0)
Non-Hodgkin's lymphoma	n (%)	41 (15)	38 (14)
Multiple myeloma	n (%)	43 (15)	45 (16)
Chronic leukemia	n (%)	3 (1.1)	-
Other	n (%)	22 (7.9)	23 (8.3)
Treatment			
Remission induction chemotherapy	n (%)	119 (43)	116 (42)
Consolidation chemotherapy	n (%)	32 (12)	35 (13)
Autologous stem cell transplantation	n (%)	101 (36)	103 (37)
Allogenic stem cell transplantation	n (%)	25 (9.0)	16 (5.8)
Other	n (%)	2(0.7)	7 (2.5)
Laboratory values at randomization			
Platelet count	10^9/L, mean ± SD	87 ± 100	79 ± 75
Hemoglobin	g/L, mean ± SD	81 ± 29	82 ± 24
Activated partial thromboplastin time	s, mean ± SD	29 ± 7.9	29 ± 8.7
Prothrombin time	s, mean ± SD	12 ± 2.2	12 ± 1.9
Fibrinogen	g/L, mean ± SD	3.7 ± 1.3	3.6 ± 1.4
Medication and medical history			
Anticoagulant/antiplatelet therapy	n (%)	30 (11)	31 (11)
Bleeding	n (%)	67 (24)	72 (26)
Infection	n (%)	26 (9.3)	27 (10)
Prior platelet transfusions	n (%)	181 (67)	162 (60)
Prior red cell transfusions	n (%)	197 (71)	191 (69)
Prior stem cell transplant procedures	n (%)	22 (7.9)†	9 (3.2)
Prior pregnancies	n (%)	61 (22)	66 (24)

n = number of transfusion treatment periods; SD = Standard deviation; †$p < 0.05$

Table 2

Bleeding complications (intention to treat analysis)

		Control	Intervention
No. of transfusion treatment periods		*279*	*277*
Primary endpoint			
WHO grade 2, 3 or 4 bleeding[#]		*143 (51%)*	*150 (54%)*
No. of days from *randomization* to first grade 2, 3, or 4 bleeding	*median (IQR)*	*5 (2-8)*	*5.5 (2-9)*
Percentage of days with grade 2, 3, or 4 bleeding[$]	*median (IQR)*	*3 (0-14)*	*5 (0-15)*
No. of days with grade 2, 3, or 4 bleeding	*median (IQR)*	*1 (0-2)*	*1 (0-2)*
Bleeding details			
Highest grade of bleeding			
None or grade 1		*136 (49%)*	*127 (46%)*
Grade 2		*131 (47%)*	*139 (50%)*
Grade 3		*6 (2%)*	*5 (2%)*
Grade 4		*6 (2%)*	*6 (2%)*

WHO = world health organization; IQR=interquartile range

[#] difference: 3 percentage points, 95% CI (-6 to 11), p-value for non-inferiority 0.012

after correcting for stratification factors (center, diagnosis AML/non-AML and treatment phase conventional/stem cell):

difference: 1 percentage points, 95% CI (-6 to 9), p-value for non-inferiority 0.002

[$] p-value for superiority of mean percentages 0.535

Bleeding complications (per protocol analysis)

		Control	Intervention
No. of transfusion treatment periods		*220*	*205*
Primary endpoint			
WHO grade 2, 3 or 4 bleeding[#]		*97 (44%)*	*107 (52%)*
No. of days from *first transfusion* to first grade 2, 3, or 4 bleeding	*median (IQR)*	*3 (1-5)*	*3 (1-5)*
Percentage of days with grade 2, 3, or 4 bleeding[$]	*median (IQR)*	*0 (0-15)*	*4 (0-17)*
No. of days with grade 2, 3, or 4 bleeding	*median (IQR)*	*0 (0-2)*	*1 (0-2)*
Bleeding details			
Highest grade of bleeding			
None or grade 1		*123 (56%)*	*98 (48%)*
Grade 2		*87 (40%)*	*102 (50%)*
Grade 3		*4 (2%)*	*2 (1%)*
Grade 4		*6 (3%)*	*3 (2%)*

WHO = world health organization; IQR=interquartile range

[#] difference:8 percentage points, 95% CI (-2 to 18), p-value for non-inferiority 0.19

after correcting for stratification factors (center, diagnosis AML/non-AML and treatment phase conventional/stem cell):

difference: 10 percentage points, 95% CI (1 to 19), p-value for non-inferiority 0.29

[$] p-value for superiority of mean percentages 0.538

Table 3

Platelet transfusion increment (intention to treat)

			Control	Intervention	
No. of platelet transfusions			*1568*	*1659*	
Efficacy parameters					
CI-1 hour 10^9/L	mean ± SD		*25 ± 14 (n=848)*	*13 ± 8 (n=997)*	
CCI-1 hour	mean ± SD		*13 ± 7 (n=848)*	*8 ± 5 (n=997)*	*p-value<0.001*
CI-24 hour 10^9/L	mean ± SD		*14 ± 14 (n=953)*	*8 ± 9 (n=1007)*	
CCI-24 hour	mean ± SD		*7 ± 7 (n=953)*	*4 ± 4 (n=1007)*	*p-value<0.001*
Transfusion failure					
CCI-1 hour < 7.5	failure rate	median (IQR)	*0 (0-0.08)*	*0.5 (0.09-0.75)*	*p-value<0.001*
CCI-24 hour < 4.5	failure rate	median (IQR)	*0 (0-0.33)*	*0.50 (0.20-0.83)*	*p-value<0.001*
CCI-24 hour ≤ 0	failure rate	median (IQR)	*0 (0-0)*	*0 (0-0.08)*	*p value=0.013*

CI = Count increment; CCI = Corrected count increment; SD = Standard deviation

Platelet transfusion increment (per protocol)

			Control	Intervention	
No. of platelet transfusions			*1395*	*1391*	
Efficacy parameters					
CI-1 hour	mean ± SD 10^9/L		*25 ± 14 (n=796)*	*12 ± 8 (n=868)*	
CCI-1 hour	mean ± SD		*13 ± 7 (n=796)*	*7 ± 4 (n=868)*	*p-value<0.001*
CI-24 hour	mean ± SD 10^9/L		*14 ± 14 (n=895)*	*7 ± 8 (n=897)*	
CCI-24 hour	mean ± SD		*8 ± 7 (n=895)*	*4 ± 4 (n=897)*	*p-value<0.001*
Transfusion failure					
CCI-1 hour < 7.5	failure rate	median (IQR)	*0 (0-0.02)*	*0.50 (0.16-0.89)*	*p-value<0.001*
CCI-24 hour < 4.5	failure rate	median (IQR)	*0 (0-0.33)*	*0.50 (0.18-0.93)*	*p-value<0.001*
CCI-24 hour ≤ 0	failure rate	median (IQR)	*0 (0-0)*	*0 (0-0.02)*	*p value=0.014*

CI = Count increment; CCI = Corrected count increment; SD = Standard deviation

Table 4.

Transfusion requirement (intention to treat).

		Control	Intervention	
No. of transfusion treatment periods		*279*	*277*	
No. of red cell transfusions	median (IQR)	*4 (2-7)*	*4 (2-6)*	*p-value=0.135*
No. of plasma transfusions	median (IQR)	*0 (0-0)*	*0 (0-0)*	*p-value=0.842*
PLT transfusion interval[1]	mean hours (95% CI)	*83 (77-91)*	*71 (67-77)*	*p-value=0.002*
No. of PLT transfusions per transfusion treatment period	median (IQR)	*4 (2-7)*	*5 (2.5-7.5)*	*p value=0.328*

IQR = Interquartile range; PLT = Platelet; SD = Standard deviation; [1]using all treatment periods via mixed Poisson model

Transfusion requirement (per protocol).

		Control	Intervention	
No. of transfusion treatment periods		*220*	*205*	
No. of red cell transfusions	median (IQR)	*3 (2-6)*	*3 (2-5)*	*p-value=0.34*
No. of plasma transfusions	median (IQR)	*0 (0-0)*	*0 (0-0)*	*p-value=0.59*
PLT transfusion interval[1]	mean hours (95% CI)	*91 (83-100)*	*71 (67-77)*	*p-value<0.001*
No. of PLT transfusions per transfusion treatment period	median (IQR)	*3 (2-6.75)*	*5 (3-7.5)*	*p-value=0.085*

IQR = Interquartile range; PLT = Platelet; SD = Standard deviation; [1]using all treatment periods via mixed Poisson model)

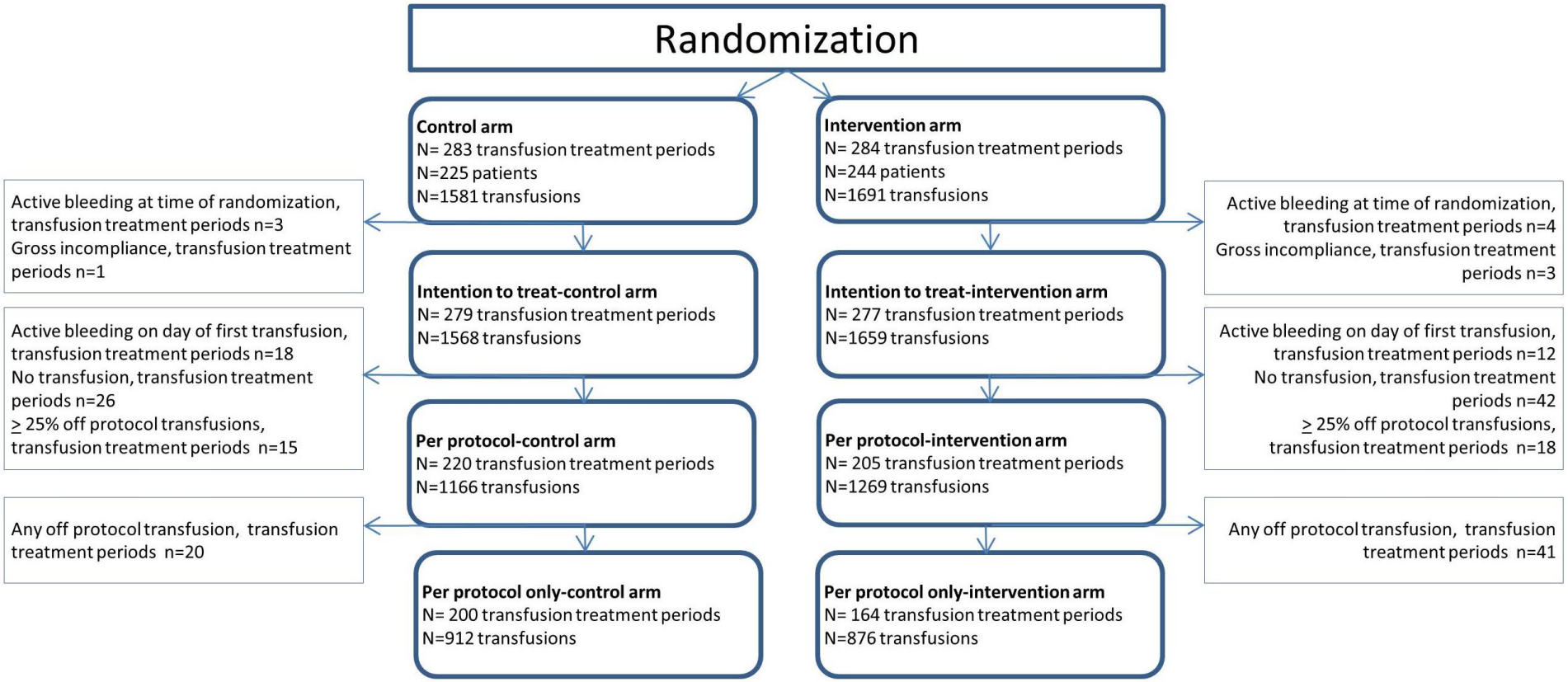
Figure legends

Figure 1. Flow diagram of the study. In total, 567 randomizations occurred in 469 patients. The intention-to-treat analysis set consisted of all transfusion treatment periods in which the patient met the in- and exclusion criteria. In the event of >25% off-protocol transfusions or no transfusions, these episodes were analyzed 'as randomized'. For the intention-to-treat analysis, the first day of observation was the day of randomization. The per-protocol set consisted of all 'on-protocol' episodes, i.e. episodes in which the percentage of off-protocol transfusions exceeded 25% before the first ≥ grade 2 bleeding event or episodes without transfusions were excluded. For the per-protocol analysis, the first day of observation was the day of the first platelet transfusion. The per-protocol-only analysis set consisted of all transfusion treatment periods in which only on-protocol transfusions are administered before a grade ≥2 bleeding occurred; the first day of observation was the day of the first platelet transfusion.

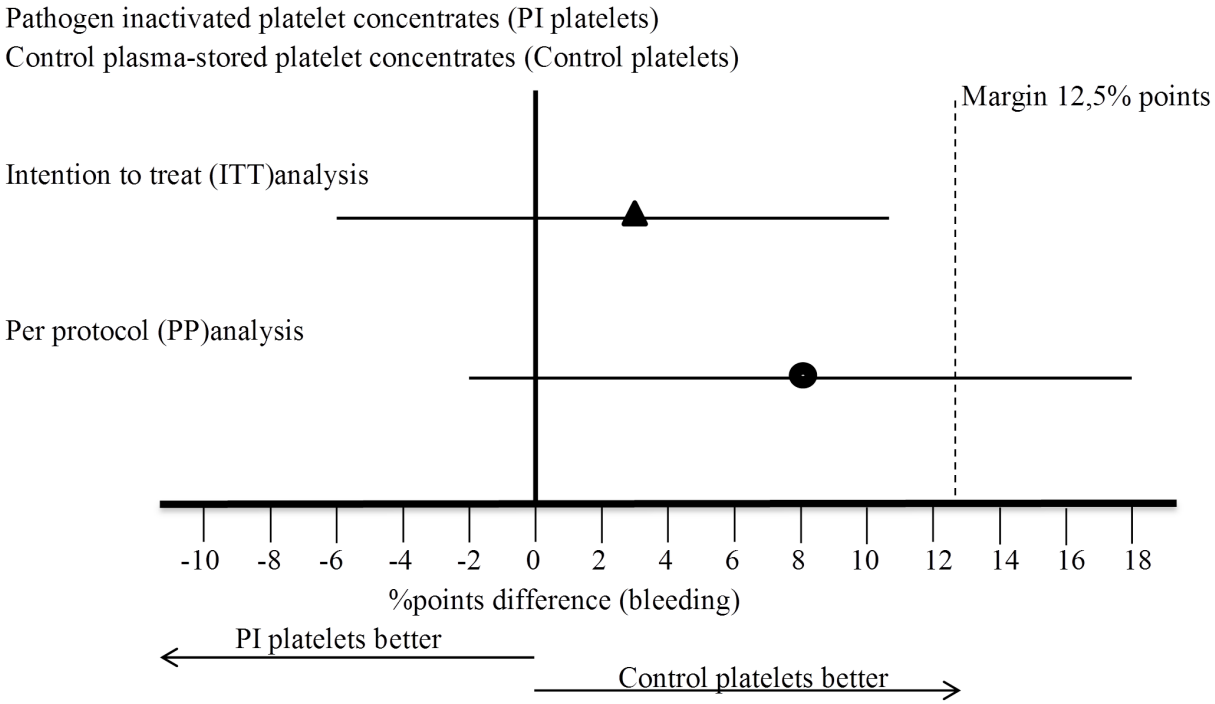
Figuur 2. Non inferiority plot comparing the difference in percentage of transfusion treatment periods with world health organization grade 2,3,4 bleeding in the intervention – and control –arm. The point estimates of the difference in percentage points and their 95% confidence intervals are displayed for the intention- to- treat analysis and the per –protocol analysis. The dotted vertical line shows the predefined margin of 12.5 percentage points. For the intention –to-treat analysis the non- inferiority criterion is met. For the per-protocol analysis the 95% confidence interval exceeds the margin of 12.5% points, the non- inferiority criterion is not satisfied.

Figure 3. Kaplan - Meier analysis HLA-class I alloimmunization. This figure shows the time to the appearance of HLA-class I alloantibodies in the PPO population (i.e. a signal higher than 5SD above the normalized background signal in the Luminex assay).

Figure 1. Flow diagram of the study.



Figuur 2. Non inferiority plot comparing the difference in percentage of transfusion treatment periods with world health organization grade 2,3,4 bleeding in the intervention – and control – arm.



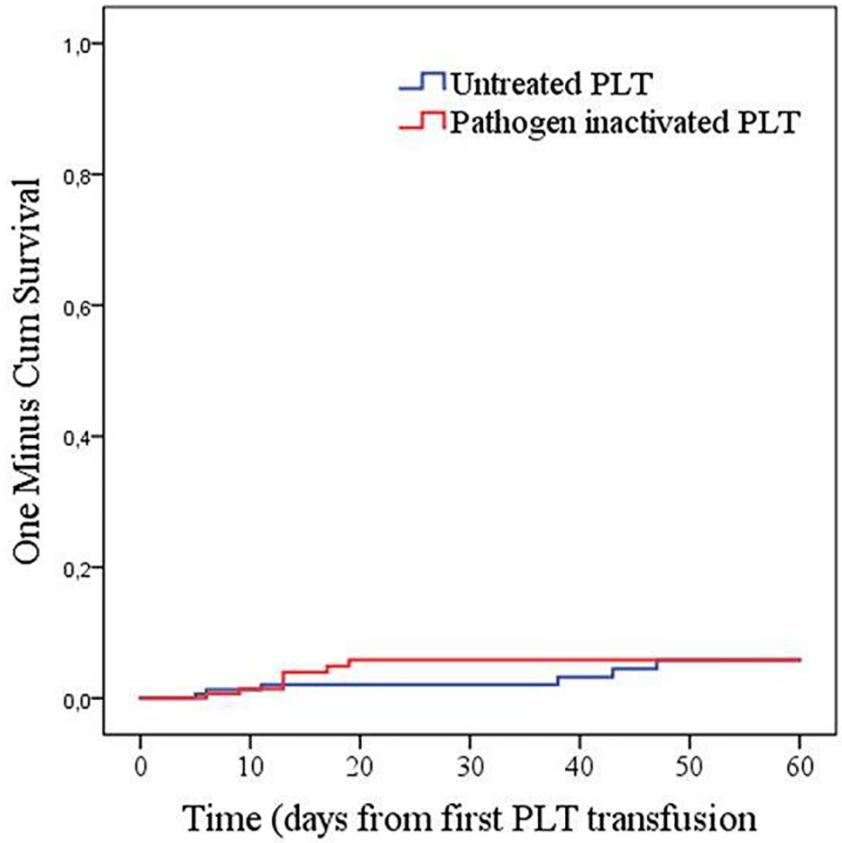


Figure 3. Kaplan-Meier analysis HLA alloimmunization.